## REMARKS

Claims 124-150 are pending in the present application. It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

### I. Objections to Claims 124 and 131

Claim 124 is objected to because of the following informalities: the term "comprises a modification of a cyclohexadepsipeptide synthetase gene" should be "comprises a modification in a ... gene" or "comprises a modified ... gene." Claim 24 was amended to recite "comprises a a disruption or a deletion in a ... gene."

Claim 131 is objected to because of the recitation of "parent filamentous fungal cell" and while *Fusarium venenatum* is a filamentous fungal cell, for clarity and consistency, the Office Action suggests that the term be amended to recite "parent *Fusarium venenatum* cell". Claim 131 was amended to recite "parent *Fusarium venenatum* cell".

Applicants submit that the objections have been overcome and respectfully request withdrawal of the objections.

### II. Rejection of Claims 124-150 under 35 U.S.C. § 112, Second Paragraph

Claims 124-150 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several grounds.

Ground 1: The Office Action states that claims 124, 137-139, and 150 (claims 125-136, 140-149 dependent thereon) are indefinite in the recitation of "nucleic acid sequence encoding the ... polypeptide", "nucleic acid sequence encodes a protease", or "nucleic acid sequence encodes an enzyme selected..." because it is unclear as to how a sequence can encode a molecule. The Office Action suggests that the terms be amended to recite "nucleic acid encoding the ... polypeptide", "nucleic acid encodes a protease or "nucleic acid encodes an enzyme selected..." Applicants have amended the above claims in accordance with the Office Action's suggestion.

Ground 2: The Office Action states that claims 124 and 139 (claims 125-138 and 140-150 dependent thereon) are indefinite in the recitation of "(ii) the mutant cell comprises a second nucleic acid sequence which comprises a modification of a cyclohexadepsipeptide synthetase gene" because (1) it is unclear as to how a cell, which is an organism, can comprise a

sequence, and (2) it is unclear as to how a sequence can comprise a modification of a gene. Claim 124 was amended to recite "the mutant cell comprises a second nucleic acid which comprises a disruption or a deletion in a cyclohexadepsipeptide synthetase gene" while claim 139 was amended to recite "a second nucleic acid comprising a disruption or a deletion in a cyclohexadepsipeptide synthetase gene."

Ground 3: The Office Action states that claim 131 is indefinite in the recitation of "at least about 25%" because use of this language is contradictory since the term "about" can be interpreted as "less than" whereas the term "at least" is synonym of "no less than". Claim 131 was amended to recite "at least 25%".

Ground 4: The Office Action states that claims 128 and 143 are indefinite in the recitation of "a cyclohexadepsipeptide synthetase which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO: 1, (ii) the cDNA sequence of SEQ ID NO: 1, or (iii) a complementary strand of (i), (ii) or (iii)" because (1) (a) a protein is not encoded by a sequence but rather by a nucleic acid, (b) it is unclear how a sequence can hybridize to another sequence since, as known in the art, hybridization takes place between nucleic acid molecules, and (c) "medium stringency conditions" is indefinite absent a statement indicating the hybridization/wash conditions which correspond to "medium stringency"; (2) the term "a complementary strand of (i)...(iii) is indefinite since it is unclear which complements are encompassed by the claims and if the intended complement is the entire complementary strand, the term should be amended to recite "complete complementary strand"; and (3) the term "(iii) complementary strand of (i), (ii) or (iii)" is indefinite since it is unclear as to how (iii) can be a complement of itself. Applicants have amended claims 128 and 143 in accordance with the Office Action's suggestions.

Ground 5. The Office Action states that claims 126, 127, 141, and 142 are indefinite in the recitation of "morphological mutant" as it is unclear what the meaning of the term is within the context of the claims and the specification does not describe the term either. This rejection is respectfully traversed. Applicants note that on page 5, lines 8-10, the specification states: "In another more preferred embodiment, the *Fusarium venenatum* cell is a morphological mutant of *Fusarium venenatum* A3/5 or *Fusarium venenatum* ATCC 20334, as disclosed in WO 97/26330." WO 97/26330 defines the term "morphological mutant."

Ground 6: The Office Action states that claim 133 is indefinite in the recitation of "cell comprises at least two copies of the first nucleic acid sequence" since it is unclear as to how a cell can comprise a sequence since a cell is a microorganism and a sequence is a graphical representation of a nucleic acid or a polypeptide, as discussed above. Applicants have

amended claim 133 to recite "the mutant cell comprises at least two copies of the first nucleic acid".

Ground 7: The Office Action states that claims 136 and 149 (claims 137, 138, 150 dependent thereon) are indefinite in the recitation of "cell further comprises one or more third nucleic acid sequences" because it is unclear as to how one can have more than one nucleic acid sequence being "third", and if the intended meaning of the term is a "cell further comprises one or more nucleic acids in addition to the two nucleic acids already present in the mutant cell of the method of claims 124 or 139", the claim should be amended accordingly. The Office Action suggests that dependent claims which further limit the additional nucleic acid may recite "wherein a third nucleic acid..." Applicants have amended claims 136 and 149 in accordance with the Office Action's suggestions.

Ground 7: The Office Action states that claim 146 is indefinite in the recitation of "the mutant cell of claim 139 wherein the *Fusarium venenaturn* cell comprises..." as it is unclear if the limitation refers to the mutant *Fusarium venenaturn* cell or the parent *Fusarium venenatum* cell. Claim 146 was amended so the limitation refers to the mutant *Fusarium venenaturn* cell.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112, second paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection.

## III. Rejection of Claims 124-128, 131-143, and 146-150 under 35 U.S.C. § 112, First Paragraph

Claims 124-128, 131-143, and 146-150 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Office Action states:

> While the specification discloses the *F. venenatum* cyclohexadepsipeptide synthetase of SEQ ID NO: 2 and its corresponding nucleic acid (SEQ ID NO: 1), there is no disclosure of other cyclohexadepsipeptide synthetase genes from other organisms as encompassed by the claims. Also, there is no disclosure of the critical structural elements required in a polynucleotide to encode a cyclohexadepsipeptide synthetase nor is there disclosure of the structural elements related to cyclohexadepsipeptide synthetase function required in a polynucleotide which hybridizes under any conditions to a polynucleotide which encodes a 70% sequence homolog of the polypeptide of SEQ ID NO: 2. Furthermore, with the exception of a deletion in the polynucleotide of SEQ ID NO: 1, there is no disclosure of additional mutations in a *Fusarium venenatum* cell which would result in any reduction in the production of cyclohexadepsipeptides, including a 25% reduction, as recited. Similarly, with the exception of a deletion in the polynucleotide of SEQ ID NO: 1, there is no disclosure of other modifications in a cyclohexadepsipeptide synthetase gene.

The rejections are respectfully traversed.

The present invention is directed to methods for producing a secreted heterologous polypeptide, comprising: (a) cultivating a mutant cell of a parent *Fusarium venenatum* cell under conditions conducive for the production of the secreted heterologous polypeptide, wherein (i) the mutant cell comprises a first nucleic acid encoding the secreted heterologous polypeptide, and (ii) the mutant cell comprises a second nucleic acid which comprises a disruption or a deletion in a cyclohexadepsipeptide synthetase gene, wherein the mutant cell produces less cyclohexadepsipeptide than the parent *Fusarium venenatum* cell when cultured under the same conditions as a result of the disruption or the deletion in the cyclohexadepsipeptide synthetase gene; and (b) isolating the secreted heterologous polypeptide from the cultivation medium. The present invention also relates to cyclohexadepsipeptide-deficient mutant cells of a parent *Fusarium venenatum* cell, comprising (i) a first nucleic acid encoding a secreted heterologous polypeptide, and (ii) a second nucleic acid comprising a disruption or a deletion in a cyclohexadepsipeptide synthetase gene, wherein the *Fusarium venenatum* mutant cell produces less cyclohexadepsipeptide than the parent *Fusarium venenatum* cell when cultured under the same conditions as a result of the disruption or the deletion in the cyclohexadepsipeptide synthetase gene.

The Office Action asserts that while the specification discloses the *Fusarium venenatum* cyclohexadepsipeptide synthetase of SEQ ID NO: 2 and its corresponding nucleic acid (SEQ ID NO: 1), there is no disclosure of other cyclohexadepsipeptide synthetase genes from other organisms as encompassed by the claims. The Office Action further asserts that since the specification only discloses a single species of the genera of genes, one skilled in the art cannot reasonably conclude that Applicants had possession of the claimed invention at the time the instant application was filed. We respectfullly disagree with these assertions.

Applicants assert that limiting the claims to SEQ ID NOs:1 and 2 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another another *Fusarium venenatum* strain comprising a similar cyclohexadepsipeptide synthetase gene to that of SEQ ID NO: 1 and thereby attempt to circumvent the literal scope of Applicants' patent rights. Thus, a competitor seeking to avoid infringing the claims would merely have to follow the disclosure in the subsequently-issued patent to find a substitute.

Applicants assert that the description as a whole is sufficient to evidence possession of the claimed invention. Applicants have provided a detailed written description for isolating the claimed cyclohexadepsipeptide synthetase genes and preparing and probing DNA libraries (see

Example 1-4 of the specification); for determining cross-hybridization of the nucleic acids encoding the cyclohexadepsipeptide synthetase using SEQ ID NO: 1, or its complementary nucleotides (see page 20, line 20, to page 22, line 27, of the specification); for comparing the percent identity of the deduced amino acid sequence of the cyclohexadepsipeptide synthetase to the amino acid sequence of SEQ ID NO: 2 using the Clustal method according to Higgins, 1989, *CABIOS* 5: 151-153 (see page 18, line 31, to page 19, line 6, of the specification); and for characterizing the activity of the cyclohexadepsipeptide synthetase (see page 8, lines 4-14, of the specification). Applicants assert, therefore, that it is well within the skill in the art to practice the invention using the Applicants' description. On the basis of Applicants' disclosure, one skilled in the art would know how to identify and isolate such cyclohexadepsipeptide synthetase genes. One of ordinary skill in the art would recognize that the above-noted methods allows the isolation and identification of genes encoding polypeptides having cyclohexadepsipeptide synthetase activity.

The Office Action also asserts that with the exception of a deletion in the polynucleotide of SEQ ID NO: 1, there is no disclosure of additional mutations in a *Fusarium venenatum* cell which would result in any reduction in the production of cyclohexadepsipeptides, including a 25% reduction, as recited. Similarly, with the exception of a deletion in the polynucleotide of SEQ ID NO: 1, there is no disclosure of other modifications in a cyclohexadepsipeptide synthetase gene. We respectfuly disagree with the Office Action's premise in practicing the instant invention.

Applicants have shown that the deduced amino acid sequence (SEQ ID NO: 2) of the cyclohexadepsipeptide synthetase gene of SEQ ID NO: 1 shares approximately 59% identity with the deduced amino acid sequence of the cyclohexadepsipeptide synthetase gene (*esyn1*) of *Fusarium scirpi* (Haese *et al.*, 1993, *Mol. Microbiol.* 7: 905-914; DNA sequence listed in EMBL database under accession number Z18755). This sequence comparison indicated there are regions of conserved homology between the sequences at the DNA level, which can be used to construct a disruption or deletion vector for use in another *Fusarium venenatum* cell without any knowledge of the DNA sequence in that cell. Applicants have provided evidence of this state of art by reference to Herrmann *et al.* (*Molecular Plant-Microbe Interactions* 9: 226-232, 1996) who showed that an internal fragment of the *Fusarium scirpi* cyclohexadepsipeptide synthetase gene was useful in disrupting the *Fusarium avenaceum* cyclohexadepsipeptide synthetase gene without any knowledge of the full nucleic acid sequence of the *Fusarium avenaceum* gene. Consequently, Applicant's disclosure combined with the knowledge in the prior art can be used to construct a mutant cell of a *Fusarium venenatum* parent strain, wherein the mutant comprises

a disruption or a deletion in a cyclohexadepsipeptide synthetase gene, wherein the mutant cell produces less cyclohexadepsipeptide than the parent *Fusarium venenatum* cell when cultured under the same conditions as a result of the disruption or the deletion in the cyclohexadepsipeptide synthetase gene.

The Office Action also asserts that the state of the art teaches that sequence comparisons alone should not be used to determine function and that small structural changes can drastically change function. Applicants submit this argument is misplaced.

Applicants are not mutating a cyclohexadepsipeptide synthetase gene to change the function of encoded enzyme. In the instant invention, Applicant is simply disrupting or removing a portion of a cyclohexadepsipeptide synthetase gene sequence so expression of the gene is disrupted and no cyclohexadepsipeptide is produced. The mutant cell of the instant invention comprises a nucleic acid which comprises a disruption or a deletion in a cyclohexadepsipeptide synthetase gene, wherein the mutant cell produces less cyclohexadepsipeptide than the parent *Fusarium venenatum* cell when cultured under the same conditions as a result of the disruption or the deletion in the cyclohexadepsipeptide synthetase gene. Applicants agree that sequence comparisons alone should not be used to determine function. However, in the methods and mutant cells of the instant case, Applicants are claiming a deletion or a disruption of a cyclohexadepsipeptide synthetase gene, such as a gene encoding a cyclohexadepsipeptide synthetase having an amino acid sequence which has at least 70% identity with SEQ ID NO: 2; or a cyclohexadepsipeptide synthetase which is encoded by a nucleic acid which hybridizes under at least medium stringency conditions with (i) the nucleic acid of SEQ ID NO: 1, (ii) the cDNA of SEQ ID NO: 1, or (iii) a complete complementary strand of (i) or (ii). Moreover, the Office Action cites no evidence that small structural changes can change the function of the encoded product of a cyclohexadepsipeptide synthetase gene. Applicants point out that a necessary step in practicing the invention is to determine the level of cyclohexadepsipeptides produced by the mutant *Fusarium venenatum* cell using the method of Visconti *et al.*, 1992, *Journal of Agriculture and Food Chemistry* 40: 1076-1082 (see page 8, lines 4-14, of the specification).

The Office Action also asserts that there is no disclosure of the critical structural elements required in a polynucleotide to encode a cyclohexadepsipeptide synthetase nor is there disclosure of the structural elements related to cyclohexadepsipeptide synthetase function required in a polynucleotide which hybridizes under any conditions to a polynucleotide which encodes a 70% sequence homolog of the polypeptide of SEQ ID NO: 2. We respectfully disagree with this assertion.

A cyclohexadepsipeptide synthetase which is encoded by a nucleic acid which hybridizes under at least medium stringency conditions with (i) the nucleic acid of SEQ ID NO: 1, (ii) the cDNA of SEQ ID NO: 1, or (iii) a complete complementary strand of (i) or (ii), as claimed herein, dictates that all species within the genus will be structurally similar and have cyclohexadepsipeptide synthetase activity. Applicants respectfully point out that the entire sequence of a cyclohexadepsipeptide synthetase gene is used as a probe in a Southern hybridization as indicated by the claim language of SEQ ID NO:1, as well as the cDNA sequence thereof, or complete complementary strands thereof. Moreover, a cyclohexadepsipeptide synthetase having an amino acid sequence which has at least 70% identity with SEQ ID NO: 2, as claimed herein, also dictates that all species within the genus will be structurally similar and have cyclohexadepsipeptide synthetase activity. Consequently, the critical structural and finctional elements required in a polynucleotide to encode a cyclohexadepsipeptide synthetase is determined by the entire nucleic acid of SEQ ID NO: 1 and the gene product of SEQ ID NO: 2.

Applicants submit that the information disclosed in the specification combined with the knowledge of the art provides sufficient guidance to inform the skilled artisan that Applicants were in possession of the claimed invention at the time the application was filed.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112, first paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection.

## IV. Rejection of Claims 124-128, 131-143, and 146-150 under 35 U.S.C. § 112, First Paragraph

Claims 124-128, 131-143, and 146-150 were rejected under 35 U.S.C. § 112, first paragraph, because the specification "does not reasonably provide enablement for (1) a method for producing a secreted heterologous protein using any mutant *Fusarium venenatum* cell which has been modified in any way to produce less cyclohexadepsipeptide synthetase and further comprises any cyclohexadepsipeptide synthetase gene, any cyclohexadepsipeptide synthetase polynucleotide encoding a 70% sequence homolog of the polypeptide of SEQ ID NO: 2, or any cyclohexadepsipeptide synthetase polynucleotide which hybridizes under any conditions to a polynucleotide encoding a 70% sequence homolog of the polypeptide of SEQ ID NO: 2, wherein said gene or polynucleotide has been modified in any way, or (2) any mutant *Fusarium venenatum* cell which has been modified in any way to produce less cyclohexadepsipeptide synthetase and further comprises any cyclohexadepsipeptide synthetase gene, any cyclohexadepsipeptide synthetase polynucleotide encoding a 70% sequence homolog of the

polypeptide of SEQ ID NO: 2, or any cyclohexadepsipeptide synthetase polynucleotide which hybridizes under any conditions to a polynucleotide encoding a 70% sequence homolog of the polypeptide of SEQ ID NO: 2." The Office Action also states:

> [D]ue to the lack of relevant examples, the amount of information provided, the lack of knowledge about the critical structural elements required to display the desired function, and the unpredictability of the prior art in regard to annotation of function based on structural homology, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to (1) isolate other cyclohexadepsipeptide synthetase genes, (2) isolate and test polynucleotides encoding polypeptides having at least 70% sequence identity to the polypeptide of SEQ ID NO: 2, (3) isolate and test polynucleotides which hybridize under any conditions to polynucleotides encoding polypeptides having at least 70% sequence identity to the polypeptide of SEQ ID NO: 2, and (4) find other modifications in a *Fusarium* cell or in a cyclohexadepsipeptide synthetase gene which would result in less production of cyclohexadepsipeptides.

These rejections are respectfully traversed.

Applicants submit that undue experimentation would not be required to practice the invention because Applicants' disclosure provides considerable direction and guidance on how to practice their invention and presents sufficient working examples. There was a high level of skill in the art at the time when the application was filed, and Applicants' enabling disclosure in combination with that skill in the art provides sufficient knowledge needed to practice the invention.

In addition to the arguments provided in Section III above, Applicants provide the following evidence

Applicants' specification provides an enabling description as to how to produce mutant cells from a parent *Fusarium venenatum* cell by deleting or disrupting a nucleic acid sequence encoding a cyclohexadepsipeptide synthetase in the parent cell (see page 5, line 14 to page 8, line 3 and Examples 5 and 6), and how to express a secreted heterologous protein in such a mutant cell (page 11, line 22, to page 17, line 24).

Applicants' specification also provides an enabling description as to how to isolate other cyclohexadepsipeptide synthetase genes on page 18, line 8 to page 28, line 9. A person of skill in the art, with the accession numbers NRRL B-30068-30070 provided in the specification, can obtain the claimed sequence of SEQ ID NO:1 from the Agricultural Research Service Patent Culture Collection and follow the appropriate techniques described in Applicants' specification to excise the nucleotide sequence from the deposited organisms. Applicants have also provided detailed instructions on page 20, line 20, to page 22, line 27, of the specification, for performing standard Southern hybridization under at least medium stringency conditions to identify genes encoding cyclohexadepsipeptide synthetases from other strains of different genera or species.

Applicants disclose the following probes on page 5, line 26, to page 6, line 5, of the specification for use in conducting the hybridization: the nucleic acid sequence of SEQ ID NO:1 or its complementary strand; or a subsequence thereof which encodes a polypeptide fragment which has cyclohexadepsipeptide synthetase activity. The use of these nucleotide sequences as probes enables the identification and isolation of other genes that encode polypeptides having cyclohexadepsipeptide synthetase activity, such as genes which are closely related or essentially identical to the gene of SEQ ID NO:1 encoding a polypeptide having cyclohexadepsipeptide synthetase activity.

Once a gene is isolated and its nucleotide sequence determined, the nucleotide sequence and the deduced amino acid sequence thereof can then be compared to SEQ ID NO:1 and SEQ ID NO:2, respectively, to ascertain whether the gene falls within the scope of the instant claims. Applicant has detailed on page 18, line 31, to page 19, line 6, of the specification, instructions for determining the degree of identity between two amino acid sequences by the Clustal method (Higgins, 1989, *CABIOS* 5: 151-153) using the LASERGENE™ MEGALIGN™ software (DNASTAR, Inc., Madison, WI) with the multiple alignment parameters of gap penalty of 10, and gap length penalty of 10 and pairwise alignment parameters of Ktuple=1, gap penalty=3, windows=5, and diagonals=5, and on page 26, lines 23-29, of the specification, instructions for determining the degree of homology between two nucleic acid sequences by the Wilbur-Lipman method (Wilbur and Lipman, 1983, *Proceedings of the National Academy of Science USA* 80: 726-730) using the LASERGENE™ MEGALIGN™ software (DNASTAR, Inc., Madison, WI) with the multiple alignment parameters of gap penalty of 10 and gap length penalty of 10 and pairwise alignment parameters of Ktuple=3, gap penalty=3, and windows=20. These methods are highly predictable and do not require undue experimentation.

With the information provided by Applicants in the specification and the knowledge available in the pertinent art, one skilled in the art can construct disruption or deletion vectors for transformation into any *Fusarium venenatum* cell, shown to produce cyclohexadepsipeptide, to disrupt or delete a cyclohexadepsipeptide synthetase gene without knowledge of the gene's sequence. For example, a DNA fragment containing a conserved or homologous region interrupted with a selectable marker or a DNA fragment with a portion of the conserved or homologous region removed by digestion with a restriction enzyme can be used with reasonable predictability to replace the corresponding similar gene via homologous recombination in a *Fusarium venenatum* cell that produces cyclohexadepsipeptide.

Applicants assert, therefore, that it is well within the skill of the art to make

cyclohexadepsipeptide-deficient *Fusarium venenatum* cells using the nucleic acid sequences disclosed in the specification and the prior art without being provided with the corresponding DNA sequences encoding the enzymes involved in the biosynthesis of cyclohexadepsipeptide. The need for isolation of the gene of a *Fusarium venenatum* cell, delineation of the nucleic acid sequence, and a determination of which modifications would lead to deficient production of cyclohexadepsipeptide is not necessary to disrupt or delete a gene involved in the biosynthesis of cyclohexadepsipeptide.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112, first paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection.

## V. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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